UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/019,199	12/20/2001	Norbert Maurer	INEX.P-005	6234

21121 7590 11/30/2004

OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

EXAMINER	
KISHORE, GOLLAMUDI S	
ART UNIT	**PAPER NUMBER**
1615	

DATE MAILED: 11/30/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/019,199
Filing Date: December 20, 2001
Appellant(s): MAURER ET AL.

Carl Oppedahl
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 9-13-04.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

All claims stand or fall together with regard to the rejections 1 and 2 based on Hope and claims 13-20 and 25-32 stand or fall together with regard to rejection 3 based on Schubert.

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

6,447,800	HOPE	9-2002
5,976,567	WHEELER	11-1999
6,365,179	ZALIPSKY	4-2002
WO 98/51278	INEX PHARMACEUTICALS CORPORATION	11-1998

Malone R.W., et al, "Cationic liposome-mediated RNA transfection", PNAS, vol. 86, (August 1989), pp. 6077-6081.

Schubert R., et al. "Loading of preformed liposomes with high trapping efficiency by detergent-induced formation of transient membrane holes", Chemistry and Physics of Lipids, vol. 58, (1991), pp. 121-129.

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

1. Claims 13-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hope (6,447,800) in view of Wheeler (5,976,567) or WO 98/51278.

Hope discloses a method of preparation of liposomes containing a variety of active agents. The method involves combining already formed liposomes with an active agent and organic solvent, ethanol (at least 10 %), allowing a certain amount of time and diluting the organic solvent in the external phase. The presence of organic solvent according to Hope increases the permeability of the membrane (without disrupting the liposomes) and when the organic solvent is diluted, the permeability decreases (note

col. 7, lines 32-65; Examples and claims). What are lacking in Hope are the use of a cationic lipid and the teachings of the removal of the organic solvent.

Wheeler while disclosing liposomal formulations containing nucleic acids using ethanol in the method teaches that cationic lipids such as DOTAP and DOTMA are efficient carriers of negatively charged nucleic aids for transfection (note the abstract and col. 1, line 55 et seq, Examples and claims. Wheeler's compositions further include PEG derivatized phospholipids (col. 11, lines 28-32). Although Wheeler's method does not involve using preformed liposomes, it is interesting to note Wheeler's teachings on col. 2, line 16 et seq., that loading of nucleic acids into preformed liposomes is practiced in the art. Wheeler's method involves similar procedure to the claimed method, except that the preformed liposomes are not used and the method of preparation involves the removal of ethanol by art known methods such as rotary evaporation (col. 18, line 40 through col. 19, line 12).

WO 98 while teaching compositions containing DOPAP, DSPC, and cholesterol teaches that PEG derivatized lipids provide steric stabilization and prevent the aggregation of the particles; WO therefore, includes PEG-lipids such as PEG-ceramides. The buffer used in the preparations is a citrate buffer (note the abstract, pages 17-19 and claims).

The use of cationic lipids in the method of Hope, if the active agent involves a nucleic acid would have been obvious to one of ordinary skill in the art since Wheeler teaches that cationic lipids are efficient in transfecting cells with nucleic acids in a similar method of preparation involving ethanol. The removal of the already diluted ethanol in the external medium of Hope if it is deemed undesirable would have been obvious to one of ordinary skill in the art since Wheeler shows that the external ethanol

can be removed by art known methods. The criticality of citrate buffer is not readily apparent to the examiner since the selection of the buffer depends upon the desired pH.

The inclusion of PEG-lipids in the compositions of Hope would have been obvious to one of ordinary skill in the art since WO teaches their ability to provide steric stabilization. The use of citrate buffer would have been obvious to one of ordinary skill in the art since WO teaches it is a commonly used buffer in liposomal compositions.

Applicants' arguments have been fully considered, but are not found to be persuasive. Appellant argues that the examiner chooses to ignore both the express statement in the reference and the declaration from its author based upon a statement in the application (col. 10, lines 10-13 according to appellant) that acknowledges that charged species can be rendered less charged, or uncharged by adjustment of the pH or by chemical modification and that from this statement the examiner infers, without evidence or even reasoned argument, that the Hope method is taught to work for at least some charged species. Appellant further agues that the phrase in the Hope patent "conversion to less highly charged analogs" does not necessarily imply that the charged species will cross the permeabilized membrane. These arguments are not found to be persuasive. First of all, the examiner addressed this issue and did not ignore it. First of all, Hope on col. 9, lines 50-58 clearly states that his method is not limited to ionizable solutes and that by his method an uncharged or neutral species or substance that are not capable of being induced to carry a charge by protonation, cation or anion binding and the like can be loaded into the liposomes; on col. 10, lines 17-21 Hope states, "For example, charged oligonucleotides can be converted to less highly charged analogs which continue to display biological activity by methylation or conversion to the corresponding phosphorothioates, methylphosphonates and the like". Since according to the last statement by Hope since one can convert highly charged oligonucleotides to

less charged methylated derivatives which still retain the biological activity, it would have been obvious to one of ordinary skill in the art with a reasonable expectation of success that these less charged oligonucleotides can be loaded into the liposomes although in a highly charged state they do not cross the membrane since according to Hope's first statement that that either charged or uncharged substances can be loaded by this method. Therefore, Hope's observation in his patent that highly charged substances such as polynucleotides do not cross the membrane is not teaching away. It should also be noted that Hope's statement on col. 10, lines 7-10 is, "Generally, highly negatively charged species such as polynucleotides do not cross liposomal membranes permeabilized by the solvent technique disclosed herein and are loaded with low levels of efficiency". This expression, "low levels of efficiency" certainly appear to indicate to the examiner that there is some level of crossing of the highly charged polynucleotides and therefore, one would only expect this crossing to increase if the molecule is methylated or converted to some other form which is less charged, but still retain the biological activity. As already pointed out before, Hope's subsequent statements referred to by applicants pertain to his improvement over the prior art method whereby one can load even the neutral molecules. Furthermore, it should be pointed out that instant claims are not limited just to polynucleotides. Applicants argue that Hope is an inventor on both secondary references as well and thus, it may be presumed that he was fully aware of the formulations containing cationic liposomes at the time the Hope's patent was filed. This argument is not pertinent since the same rationale will be applicable since even Peter R. Cullis one of the inventors in instant application is also one of the inventors of patent 5,976,567 (wheeler) as well as the WO reference used in the combination. Therefore, based on the above reasoning that Hope's statements in

his declaration that based on the tests he conducted during the period between 1990-1992, the Hope technique does not work with charged nucleotides is not persuasive.

Appellant's arguments that although Hope, and Wheeler methods make use of ethanol, wheeler is not used to permeabilize the pre-formed lipid membranes are not found to be persuasive. The examiner has already acknowledged that Wheeler's method does not involve the preformed liposomes. However, Wheeler's teachings on col. 2, line 16 et seq., clearly indicate that the cationic lipids have the ability to form liposomes and that a nucleic acid can be introduced into a preformed liposome and it would have been obvious to one of ordinary skill in the art from this statement coupled with his teachings that cationic lipids are very efficient in transfecting cells with nucleic acids, to use nucleic acids (polynucleotides) decreasing their charge and load by Hope's method if the desired goal is the transfection of the cells by nucleic acids. Appellant questions why one would a person skilled in the art imagine that negatively charged polynucleotides would pass through a membrane better, when that membrane contains positive charges to which they can stick as shown in Wheeler. This argument is not found to be persuasive since as pointed out above, Wheeler clearly indicates that the introduction of nucleic acids in preformed cationic liposomes can be performed and furthermore, if that were to be the rationale, since instant method uses the same cationic lipids, one would expect the same sticking. It is interesting to note Hope's statement in his declaration (# 11) that there are electrostatic interactions between for a high D:L ratio using ionizable cationic lipids according to instant invention (same lipids as in Wheeler and same sticking?).

Appellant argues that the rejection is based solely on finding the isolated elements, and the motivation to make the combination is lacking (Hope # 8). In response to applicant's arguments against the references individually, one cannot show

nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Appellant argues that the claims also contain a requirement for a modified lipid that acts as a steric barrier, and specifies that the amount of this lipid is amount effective to retard, but not prevent, aggregation of the preformed vesicles and that Hope does not disclose the use of such lipids. Appellant further argues that the examiner has not explained why one skilled in the art would think that steric stabilization is needed in the liposomes. The examiner disagrees and points out that the examiner in the final action addressed these issues. The examiner has stated that the arguments are not found to be persuasive since WO teaches on page 17 that the presence of PEG-lipid improves the formulation process by reducing aggregation of the lipid particles during formation (goal is the same as in instant invention). This aggregation will be a problem whether recognized by Hope or not since Hope's invention also involves liposomes just as in instant invention and one of ordinary skill in the art would be motivated to include PEG lipid to prevent such a aggregation during formation. Furthermore, it is well recognized in the art that the presence of PEG-lipids in liposomal formulations would extend the blood circulation time of the liposomes (please see below).

Appellant argues that the claims call for an intermediate or modified lipid that prevents aggregation without completely eliminating it and this intermediate amount is important to the success of the method of the present invention but is not suggested in the reference. Appellant points out to example seven and Hope # 17 in this regard.

These arguments are not found to be persuasive since according to instant example 7, this amount is between 2.5 to 10 mol % and applicant find that the entrapment level is increased from 45 to almost 60 % when the PEG-cer content was increased from 2.5 to 10 mol.%. According to Wheeler (fig. 46), the entrapment value is at the peak at 10 mol.% just as in instant invention. Appellant's arguments that that the ability to introduce charged therapeutic agents into a pre-formed lipid particle, without rearrangement is important and the ability to control the particle's size is important and that such control is lacking in Wheeler are not found to be persuasive since the rejection is based on Hope and Wheeler and not Wheeler alone and Hope's method clearly guides one of ordinary skill in the art in loading the active agents.

2. Claims 13-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hope (6,447,800) in view of Malone (PNAS, vol. 86, pp. 6077-6081, 1989) and Zalipsky (6,365,179).

As discussed above, Hope discloses a method of preparation of liposomes containing a variety of active agents. The method involves combining already formed liposomes with an active agent and organic solvent, ethanol (at least 10 %), allowing a certain amount of time and diluting the organic solvent in the external phase. The presence of organic solvent according to Hope increases the permeability of the membrane (without disrupting the liposomes) and when the organic solvent is diluted, the permeability decreases (note col. 7, lines 32-65; Examples and claims). What are lacking in Hope are the use of a cationic lipid and the teachings of the removal of the

organic solvent. What is also lacking in Hope is the use of a modified lipid such as PEG-phospholipid.

Malone teaches that the use of cationic liposomes containing DOTMA is an efficient way of RNA transfection (note the abstract and discussion).

Zalipsky while disclosing liposomal formulations teaches that modified lipids such as polymer derivatized lipids (PEG-phospholipids) extend the blood circulation time of the liposomes (col. 10, lines 28-37; col. 11, lines 12-57). The method of preparation in Zalipsky involves the use of ethanol and Zalipsky advocates the removal of ethanol by diafiltration (note Example 4 on col. 17).

The use of cationic lipid, DOTMA in the method of Hope, if the active agent involves a nucleic acid would have been obvious to one of ordinary skill in the art since Malone teaches that this cationic lipid is efficient in transfecting cells with nucleic acids. The removal of the already diluted ethanol in the external medium of Hope if it is deemed undesirable would have been obvious to one of ordinary skill in the art since Zalipski teaches that the external ethanol can be removed by diafiltration. The use of modified lipids in Hope would have been obvious to one of ordinary skill in the art since Zalipski also teaches that these lipids extend the circulation time of the liposomes. The criticality of citrate buffer is not readily apparent to the examiner since the selection of the buffer depends upon the desired pH.

Applicants' arguments have been fully considered, but are not found to be persuasive. Applicants provide no specific arguments to this rejection except to state that the rejection is deficient for the same reasons as discussed above. Therefore, the same response as above is applicable.

3. Claims 13-20, and 25-32 are rejected under 35 U.S.C. 103(a) as being unpatentable Schubert (Chemistry and Physics of Lipids, 58, 121-129, 1991) in view of

Malone (PNAS, vol. 86, pp. 6077-6081, 1989) and either Zalipsky (6,365,179) or WO 98/51278 of record.

Schubert discloses a method of loading preformed liposomes by detergent-induced (destabilizing agent-induced) formation of transient membrane holes. The method involves the incubation of the preformed liposomes with the active agent such as nucleic acids and removal of the detergent (note the abstract and Materials and Method section). What is lacking in Schubert is the use of a cationic lipid. What is also lacking in Schubert is the use of a modified lipid such as PEG-phospholipid or PEG-ceramide.

Malone teaches that the use of cationic liposomes containing DOTMA is an efficient way of RNA transfection (note the abstract and discussion).

Zalipsky while disclosing liposomal formulations teaches that modified lipids such as polymer derivatized lipids (PEG-phospholipids) extend the blood circulation time of the liposomes (col. 10, lines 28-37; col. 11, lines 12-57).

As discussed above, WO 98 while teaching compositions containing DOPAP, DSPC, cholesterol teaches that PEG derivatized lipids provide steric stabilization and prevent the aggregation of the particles; WO therefore, includes PEG-lipids such as PEG-ceramides. The buffer used in the preparations is a citrate buffer (note the abstract, pages 17-19 and claims).

The use of cationic lipid, DOTMA in the method of Schubert would have been obvious to one of ordinary skill in the art since Malone teaches that this cationic lipid is efficient in transfecting cells with nucleic acids. The use of modified lipids in Schubert would have been obvious to one of ordinary skill in the art since Zalipski also teaches that these lipids extend the circulation time of the liposomes or since WO 98 teaches their ability to sterically stabilize the particles. The criticality of citrate buffer is not readily

apparent to the examiner since the selection of the buffer depends upon the desired pH; one of ordinary skill in the art would be motivated to use citrate buffer since WO teaches that it is commonly used in liposomal preparations containing nucleic acids.

Applicants' arguments have been fully considered, but are not found to be persuasive. Applicants argue that Schubert is substantially cumulative with Hope except that a different method for opening the membrane of preformed liposomes is disclosed. According to applicants, in Schubert, sodium cholate, a bile salt is used to open the membrane and that the examiner has not indicated why a person skilled in the art would anticipate that changing the liposome structure to include a cationic lipid would allow loading of pre-formed lipids with a negatively charged oligonucleotide, without this step of membrane opening being necessary. This argument is not found to be persuasive since according to Shubert, the bile acid (detergent) enables transient membrane holes; that means it perturbs the membrane without disrupting the vesicles just as in instant method and the rationale for one of ordinary skill in the art to use a cationic lipid has been clearly set forth by the examiner; that is, cationic lipids are efficient in transfecting cells with nucleic acids as taught by Malone.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Gollamudi S Kishore, Ph.D
Primary Examiner
Art Unit 1615

GSK
November 29, 2004

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Conferees

PREMA MERTZ
PRIMARY EXAMINER

OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068